Specifying the Age-Sensitive Component of a Short-Term Memory

Cognitive decline that accompanies aging is believed to arise from alterations in several neuronal mechanisms and neural systems. In this issue of *Neuron*, Tamura and colleagues make the remarkable observation that age specifically diminishes middle-term memory in *Drosophila*, a memory phase dependent on expression of the amnesiac gene.

T.S. Eliot famously lamented the aged brain as "a dull head among windy spaces" (*Gerontion*, *The Waste Land and other Poems*, 1920). A little experimentation is often more productive than years of poetic angst, although, to be fair to the poet, the precision required for the analyses was not feasible with the experimental tools of the early 20th century. Gifted to science by Eliot's contemporary, Thomas Hunt Morgan, the fruit fly *Drosophila melanogaster* currently permits an unprecedented number of tools to be applied to analyze molecular mechanisms as well as anatomical regions and temporal requirements of the neural circuitry that underlies cognitive processes and, thereby, their sensitivity to age.

Aversive Pavlovian conditioning in *Drosophila* most typically pairs a foot shock, the unconditioned stimulus (US), with a specific odor, the conditioned stimulus (CS). After a single training trial, learned memory of this association, which requires a brain structure known as the mushroom body (Heisenberg, 2003), passes through at least three mechanistically and temporally distinct phases, termed, in order of appearance, short-term memory (STM), middle-term memory (MTM), and anesthesia-resistant memory (ARM) (Quinn and Dudai, 1976; Tully et al., 1996). STM, which predominates in the first half hour after training, is sensitive to mutations in the genes encoding components of cAMP signaling, such as *dunce* (*dnc*), which encodes cAMP phosphodiesterase, and *rutabaga* (*rut*), which encodes adenylate cyclase, as well as several other genes, including *volado*, *latheo*, and *leonardo*. In contrast, and important for the ensuing discussion, it is relatively insensitive to mutations in *amnesiac* (*amn*) that specifically eliminate the second memory phase, MTM, most apparent between 30 min and 3 hr after training. The last memory phase, ARM, derives from the consolidation of early memory, lasts more than 7 hr, and, while resistant to cold shock, is disrupted by mutations in a gene called *radish* (Tully et al., 1996). It is in this genetic and behavioral context (Figure 1A) that Tamura and colleagues' study (Tamura et al., 2003 [this issue of *Neuron*]) is initiated.

Tamura et al. begin by simply asking how aging affects memory and its phases in *Drosophila*. They first demonstrate that 20- to 50-day-old flies show substantially reduced memory of aversive conditioning. Initial learning is robust in aged flies, indicating no significant impairment in odor perception or shock reactivity as well as the retention of neural circuitry and mechanisms required to associate the two stimuli. Memory, 1 hr after training, is substantially reduced to ~35%–40% of young (1- or 10-day-old) controls. However, 7 hr memory is unaffected, even in 50-day-old flies, an age at which more than 50% mortality has occurred. The apparent specificity of this age-related memory impairment (AMI) to the intermediate time period suggested the hypothesis that AMI specifically alters the same memory component lost in *amnesiac*, namely, MTM. This suggestion is tested by multiple experimental analyses, each designed to isolate and measure one or other memory component in aged (20 day) flies.

If AMI is specific to MTM, then three predictions are made. First, ARM should be normal in aged flies; second, AMI should remain significant in mutants that affect STM; and finally, AMI should be invisible in *amnesiac* mutants. Tamura et al. test these predictions and find them to be true.

Anesthesia-resistant memory, ARM, may be isolated as 3 hr memory resistant to erasure by a cold shock applied 2 hr after initial training. This parameter is indistinguishable in young and aged *Drosophila*. If not ARM, then, is the age-sensitive component STM? Mutants either in STM or initial memory acquisition, *latheo*, *volado*, and *linotte*, show robust AMI: thus, these mutants do not eliminate the age-sensitive component of memory. Together these observations are consistent with a model in which a memory component other than STM (or ARM) is affected by age. As expected from this chain of logic, this memory component is shown to be MTM. Most clearly, in three different mutant alleles of *amnesiac*, residual 1 hr memory (composed of STM and ARM) is not affected by age. Thus, the age-sensitive component of memory has been eliminated in *amnesiac* mutants. Further controls show that an *amnesiac* transgene can provide all of the functions required to restore normal levels of AMI. The conclusion, then, that age specifically affects *amnesiac*-dependent MTM, is strong and has relatively few minor caveats.

First, because individual signaling proteins may function during different memory phases, mutations that affect memory may not cleanly separate different components of memory. For instance, the very low levels of 1 hr memory in *rut* mutants (which make AMI very hard to measure) are consistent with a model in which *rut* mutants have defects in multiple phases of memory. Similarly, the exclusivity of *amn* for MTM (do *amn* mutants also cause slight changes in STM?) is almost impossible to establish by genetics and behavioral studies. While exclusivity remains to be demonstrated, the data make a clear case for selectivity. Tamura et al. make it abundantly clear that age selectively reduces a specific, early component of protein synthesis-independent memory that is also selectively lost in *amn* mutants. A new finding in this study is that ARM can form under conditions when MTM is either substantially reduced or absent (Figure 1B). Whether MTM and *amnesiac* are required for the consolidation of protein synthesis-dependent long-term memory (LTM) is yet to be established; thus, it is of obvious interest to know whether *amnesiac* mutants and aged flies show reduced long-term memory.

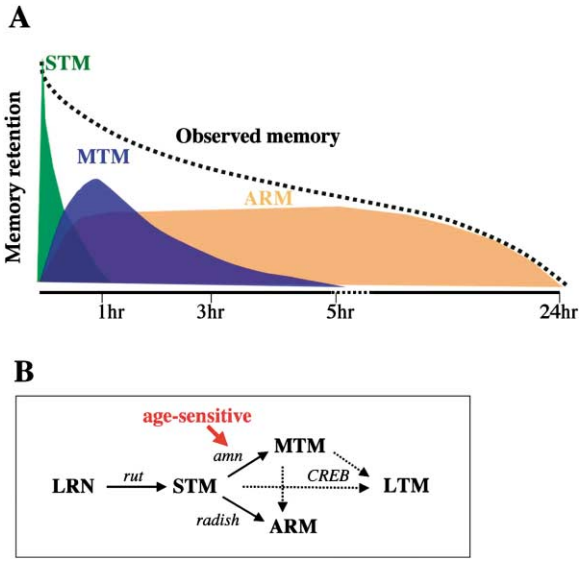

Figure 1. Phases of *Drosophila* Memory Defined by Genetics and Age

The age-sensitive phase of memory shown in the context of (A) distinct memory phases and (B) a slightly revised model for genetic dissection of olfactory memory (Tully et al., 1996). (A) In *Drosophila*, initial acquisition of aversive conditioning to an odor (also known as learning [LRN]) is followed by three distinct memory phases: short-term memory (STM) and medium-term memory (MTM), which are erased by a cold shock (anesthesia), and anesthesia-resistant memory (ARM), which is more long lasting (Tully et al., 1996). (B) In addition to showing that age specifically affects MTM, a memory phase that requires the function of the *amnesiac* (*amn*) gene, Tamura et al. (2003) show that ARM can form in the absence of MTM; thus, ARM can arise by consolidation of STM. This observation also highlights the need for further experiments to ask if formation of protein and CREB-dependent long-term memory is dependent on *amn* or MTM and whether LTM is sensitive to age.

The term "genetic dissection," beloved to *Drosophila* biologists (a Medline search indicates that this term occurs in 215 *Drosophila* papers compared to 28 in *C. elegans*), has resonated poorly in a field more accustomed to interpreting behavior in terms of brain anatomy, circuitry, or physiology. The mystical element associated with a "genetic dissection" of memory phases would be substantially dispelled if it could be shown that processes mediated by genes affecting different memory phases occur either in a specific temporal sequence or in anatomically distinct regions of the brain. Previous analyses of *amnesiac* and of mushroom body function in memory indicate that this may indeed be true.

The *amn* locus encodes a polypeptide that may be cleaved to generate three peptides: one homologous to a neuropeptide, termed PACAP, another similar to human growth hormone releasing hormone, and the third a novel molecule. While the *rut*-encoded adenylate cyclase is preferentially expressed in the mushroom body where it functions for formation olfactory associative memory (Zars et al., 2000), *amnesiac* is highly selectively expressed and functions for memory, in two dorsal paired medial (DPM) neurons outside the mushroom body (Waddell et al., 2000). Importantly, these neurons form synapses onto mushroom body lobes, generally believed to be the axonal (output) regions of the mushroom body (see Strausfeld et al., 2003, for a more complex view). Because previous elegant experiments using targeted expression of a conditional, dominant mutation in presynaptic function (Kitamoto, 2001) have shown that initial memory storage does not require mushroom body output (Dubnau et al., 2001; McGuire et al., 2001), the data are consistent with a model in which early Hebbian learning occurs in dendritic (input) regions of the mushroom body. Thus, *amnesiac* functions to modulate output from mushroom body neurons to a different field of cells in which middle-term memory may be formed or reside. This *amnesiac*-modulated process, likely to be temporally and anatomically distinct from the initial odor shock association (STM), is shown by Tamura et al. to be most sensitive to age.

How may age affect the amnesiac-dependent process? Given the relatively early understanding of how *amnesiac* functions, the role of DPM neurons, and indeed of how memory is encoded and stored, there is yet no satisfactory answer to this question, but some simple mechanisms are excluded. Aged flies lacking MTM continue to have normal levels of *amnesiac* expressed in their DPM neurons; thus DPM cell loss and reduced *amn* expression cannot be the answer. A curious observation that DPM neurons in old flies have substantially denser innervation of the mushroom body lobes stops well short of an answer but suggests promising avenues for further investigation. Is this expansion the cause of AMI? A consequence? Or simply an independent, accompanying phenomenon?

As observed at the beginning of this Preview, mechanisms of age-related memory impairment are limited by our understanding of memory mechanisms themselves. In this specific olfactory memory task, age appears to affect one phase of memory formation in *Drosophila*. But will this be true of multiple memory tasks in other organisms? Recent studies of fear conditioning (tone shock association) in the rodent amygdala indicate a role for a local neuropeptide-mediated feedback loop in setting the threshold for LTP and memory (Shumyatsky et al., 2002). Based on two examples, should one expect that an independent neuromodulatory input will prove a general feature of memory consolidation in different memory systems? Are such neuromodulatory circuits particularly sensitive to age? More detailed understanding of local circuits in brain regions and of how they contribute to specific behaviors will be required before the generality of Tamura's observations can be assessed. Recent observations that PKA signaling has opposing effects on hippocampus-dependent and hippocampus-independent (prefrontal cortex-mediated) memory tasks in aged mammals (Ramos et al., 2003) indicate a long road that lies ahead before memory mechanisms and their modulation by age can be understood. As evidenced by novel analyses of memory mutants such as *amnesiac* (Tamura et al., 2003; Waddell et al., 2000) as well as parallel studies of the mushroom body and associated neural circuitry (Dubnau et al., 2001; Heisenberg, 2003; McGuire et al., 2001), it is safe to predict that important insights will continue to be provided by thoughtful work in the simple-minded *Drosophila*.

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